January 19, 1950.

Dear Max:

Series 20-21-22 Received Tuesday noon, in very good shape. We were and will be very glad to run these down, and had very little trouble in classifying them. About half a dozen were uncertain in the first streakings on EMB Lac, but gave very definite Lac v colonies after running them through EMS Lac. I haven't checked the segregants yet to make sure that there are no Lacy whatever, but I am fairly confident about them, and will classify them with respect to their other characters in the next few days. There were a couple of cultures which were almost pure Lac-, but had less than 1/200 Lacv, and I will want to be sure that none of these are included. As usual, and very important, there were no cultures with + and - but no v. This alone, with the samples so far, pretty well precludes the occurrence of complementary segregants unconfused by nuclear distribution.

The following were segregants(all Lac-)

20 : Clone 48, i.e,, 783, 784, 392, 393, 394, 198

113 (as you predicted)

21: 98, 60, 251

22: 75, Clone 25 (103, 104, 106

Every culture listed is accounted for, except possible 22-363, which I can't find.

In every case, segregants had diploid sibs. Both of the clones included lethals, but this may not be significant.

We will be more than happy to run any further series that you have time to do. I'm beginning to think, however, that H-206 is not going to be any more valuable than all the previous cultures; possibly less so because the markers are not the same as were used in most of my linkage studies. Inthink that it's pretty well settled with the present series that complementary segregations are not taking place. We should have recovered twice as many cultures with + and - but no v, than pure + or pure -, if it occurred regularly, and if none have been found in samples from which a dozen or more segregations of pure types were recorded, I strongly suspect that there will be no "second-division" cells, at least in the cultures so far sent. There are, however, two additional diploid types that probably should be pedigreed: 1) an exceptional diploid I've isolated which is heterozygous for Mal+/- . Although this shows some apparent deviation from Mendelian ratios, the fact that it is Mal+/Mal- whereas the diploids typically are Mal-/def., suggests that this one may not be deficient, i.e., heterozygous for a lethal, and there would be a better chance of picking up complementary segregations. 2) "spontaneous" heterozygotes, (from crosses not involving Het)

I don't know a great deal about such heterozygotes, but they do occur in "normal" crosses about .05% - .1% of the prototrophs. Also, I am preparing some more complex heterozygotes (Lac, Mtl, Xyl, Gal, $T6^r$, $T1^r$, and sorbitol) for some "grand final" tests for linear linkage. Until I can get these made up and in your hands, I would recommend going back to H-168, or possibly taking time off until I can complete the analysis of the present batches.

I went over your series 15 and 12, mostly checking only the peculiar ones or the segregants. I did not have time when they first arrived to go over them in detail, owing to travel, and most of the y cultures were rather difficult to score as such (i.e., they were mostly + and -) when I got to them.

12-269 and 12-270, and 12-209 were all Lac-Mal-, as you stated. I looked through 12-198 twice, and couldn't find any Lac or Mal y: the hets must have been exceedingly scarce. In view of the typical predominance of Lac- in this series, these cultures are rather peculiar, but there is nothing obvious to do with them except perhaps check their nutrition, which I shall do as soon as I can catch up to you. I'm going to take your word on the classification of the y in this set, or I never will catch up!

In the series 20, I noticed that clone 13 (i.e. 111-118) differed from the rest of the set insofar as most of them were segregating rather high proportions (30-70%) of + segregants, except for 113 which, of course, is a pure -. I don't think this is entirely meaningless, but I don't see quite what we can do with it.

Would it make the job less tedious to take a large number of two-generation clones rather than follow the pedigree of a single cell so far out? It has been a beautiful tour de force to do these thorough pedigrees, but practically as much information could be gotten from more, but smaller clones, without taking the chances of starting everything with a segregant, and lessening what must be the terrific strain of keeping track of everything.

I was very glad to hear Sherman's approval of a brief bacteriological paper. If you would like to use this as a vehicle for describing your technique in detail, I would be glad to leave you as sole author, as I don't think the definitive description of the technique should have my name attached to it, even if it also deals with the application to the diploids (and their "single-celledness"). But that's up to you. I do feel that the technique should be published in as full detail as possible.

I'll be sending you H-213 (the Mal+/-) fairly promptly, and the other new H-cultures as soon as I can get them.

Best of luck in your new job,

Sincerely,

Joshua Lederberg